SANTA CRUZ BIOTECHNOLOGY, INC.

ATP6AP1 siRNA (m): sc-141357

BACKGROUND

Vacuolar-type H^+-ATPase (V-ATPase) is a multisubunit enzyme responsible for acidification of eukaryotic intracellular organelles. V-ATPases pump protons against an electrochemical gradient, thereby synthesizing ATP. A peripheral V_1 domain, which is responsible for ATP hydrolysis, and an integral V_0 domain, which is responsible for proton translocation, compose the V-ATPase. Nine subunits (A-H) make up the V_1 domain and five subunits (a, d, c, c' and c'') make up the V_0 domain. ATP6AP1 (ATPase, H^+ transporting, lysosomal accessory protein 1), also known as 16A, CF2, Ac45, XAP3, ATP6S1, VATPS1 (vacuolar ATP synthase S1 accessory protein) or ATP6IP1, is a type I transmembrane, V-ATPase accessory protein that is predominantly expressed in endocrine and neuronal cells. ATP6AP1 is responsible for targeting the V-ATPase enzyme to specialized complex vacuolar systems. Via its cytoplasmic tail, ATP6AP1 interacts with subunits of the V_0 domain. The disruption of this interaction in osteoclasts results in impaired bone resorption, suggesting an important role for ATP6AP1 in proper osteoclastic bone resorption.

REFERENCES

1. Supek, F., et al. 1994. A novel accessory subunit for vacuolar H^+-ATPase from chromaffin granules. J. Biol. Chem. 269: 24102-24106.
2. Getlawi, F., et al. 1996. Chromaffin granule membrane glycoprotein IV is identical with Ac45, a membrane-integral subunit of the granule's H^+-ATPase. Neurosci. Lett. 219: 13-16.
3. Jansen, E.J., et al. 1998. Intracellular trafficking of the vacuolar H^+-ATPase accessory subunit Ac45. J. Cell Sci. 111: 2999-3006.
4. Holthuis, J.C., et al. 1999. Biosynthesis of the vacuolar H^+-ATPase accessory subunit Ac45 in *Xenopus* pituitary. Eur. J. Biochem. 262: 484-491.
5. Schoonderwoert, V.T. and Martens, G.J. 2002. Structural gene organization and evolutionary aspects of the V-ATPase accessory subunit Ac45. Biochim. Biophys. Acta 1574: 245-254.

CHROMOSOMAL LOCATION

Genetic locus: Atp6ap1 (mouse) mapping to X A7.3.

PRODUCT

ATP6AP1 siRNA (m) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ATP6AP1 shRNA Plasmid (m): sc-141357-SH and ATP6AP1 shRNA (m) Lentiviral Particles: sc-141357-V as alternate gene silencing products.

For independent verification of ATP6AP1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-141357A and sc-141357B.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ATP6AP1 siRNA (m) is recommended for the inhibition of ATP6AP1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

ATP6AP1 (E-10): sc-515607 is recommended as a control antibody for monitoring of ATP6AP1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ATP6AP1 gene expression knockdown using RT-PCR Primer: ATP6AP1 (m)-PR: sc-141357-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.